Attorney Docket No.: 5636.210-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Nielsen et al. Confirmation No: To be assigned

Serial No.: To be assigned Group Art Unit: To be assigned

Filed: December 21, 2001 Examiner: To be assigned

For: Glucoamylase Variants

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, DC 20231

Sir:

Before examination, please amend the above-identified application as follows (a marked up version pursuant to 37 C.F.R. 1.21 is attached hereto):

IN THE CLAIMS:

Please cancel claims 19-28 without prejudice or disclaimer.

Please substitute the following amended claims for the pending claims having the same claim numbers:

6. (Amended.) The variant according to claim 1, wherein the parent homologous glucoamylase is the *Aspergillus niger* G1 glucoamylase.

7. (Amended.) The variant according to claim 1, wherein the glucoamylase is a truncated glucoamylase, in particular in the C- terminal.

8. (Amended.) A DNA construct comprising a DNA sequence encoding a glucoamylase variant according to claim 1.

10. (Amended.) A cell which is transformed with a DNA construct according to claim 8.

13. (Amended.) A process for converting starch or partially hydrolyzed starch into a syrup containing dextrose, said process including the step saccharifying starch hydrolyzate in the presence of a glucoamylase variant according to claim 1.

15. (Amended.) The process of claim 13, comprising saccharification of a starch hydrolyzate of at least 30 percent by weight of dry solids.

16. (Amended.) The process of claim 13, wherein the saccharification is conducted in the presence of a debranching enzyme selected from the group of pullulanase and isoamylase, preferably a pullulanase derived from *Bacillus acidopullulyticus* or *Bacillus deramificans* or an isoamylase derived from *Pseudomonas amyloderamosa*.

17. (Amended.) The process of claim 13, wherein the saccharification is conducted at a pH of 3 to 5.5 and at a temperature of 60-80°C, preferably 63-75°C, for 24 to 72 hours, preferably for 36-48 hours at a pH from 4 to 4.5.

18. (Amended.) A method of saccharifying a liquefied starch solution, which method comprises

(i) a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent step of

(ii) one or more high temperature membrane separation steps

wherein the enzymatic saccharification is carried out using a glucoamylase variant according to claim 1.

REMARKS

This amendment is submitted to cancel claims in order to reduce the filing fee. There is no new matter added, and entry of the amendment is respectfully requested.

Applicants enclose herewith the Sequence Listing for the above-captioned application. The computer-readable form in this application is identical with that filed in Application Serial No. 09/351,814, filed July 12, 1999. In accordance with 37 CFR 1.821(e), please use the last filed computer readable form filed in that application as the computer readable form for the instant application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the computer readable form that will be used for the instant application. The content of the attached paper entitled "SEQUENCE LISTING" and of the computer readable form filed in the parent application is the same. No new matter is added.

The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

Date: January 2, 2002

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Sir:

Below is a marked-up version of the amendments made in the accompanying amendment.

IN THE CLAIMS:

Claims 6-8, 10, 13 and 15-18 have been amended as follows:

1. (Unchanged.) A variant of a parent glucoamylase comprising one or more mutation(s) in the following position(s) or region(s) in the amino acid sequence shown in NO: 2:
Region: 1-18,
Region: 19-35,
Region: 40-62,
Region: 73-80,
Region: 93-127,
Region: 170-184,
Region: 200-212,
Region: 234-246,
Region: 287-319,
Region: 334-341,
Region: 353-374,
Region: 388-414,
Region: 445-470,
and/or in a corresponding position or region in a homologous glucoamylase which displays at least 60% homology with the amino acid sequences shown in SEQ ID NO: 2, with the exception of the following substitutions: N20C, A27C, S30P, Y48W, Y50F, W52F, R54K/L, D55G/V, G57A, K108R, D112Y, Y116A/W, S119C/W/E/G/Y/P, W120H/L/F/Y, G121T/A, R122Y, P123G, Q124H, R125K, W170F, N171S, Q172N, T173G, G174C, Y175F, D176N/E, L177H/D, W178R/D, E179Q/D,

E180D/Q, V181D/A/T, N182A/D/Q/Y/S, G183K, S184H, W212F, R241K, A246C, D293E/Q, A302V, R305K, Y306F, D309N/E, Y312W, W317F, E389D/Q, H391W, A392D, A393P, N395Q, G396S, E400Q/C, Q401E, G407D, E408P, L410F, S411A/G/C/H/D, S460P.

2. (Unchanged.) The variant of claim 1, wherein the variant comprise one or more of the following mutations: A1V, T2E/P/Q/R/H/M, L3P/N, N9A, A11P/E, I18V, L19N, N20T, G23A, A24S/T, D25S/T/R, G26A, A27S/T, W28R/Y, S30T/N, G31A, A32V, D33R/K/H, S34N, S40C, T43R, T51D/S, T53D, S56A/C, V59T/A, L60A, N93T, P94V, S95N, D97S, L98P/S, S100T/D, A102S/*, N110T, V111P, D112N, E113M/A, T114S, A115Q/G, Y116F, S119A, G127A, N182E, A201D, F202L, A203L, T204K, A205R/S, V206L/N, G207N, S208H/T/D, S209T, S211P, W212N/A/T, A246T Y312Q, N313T/S/G, A353D/S, S356P/N/D, D357S, A359S, T360V, G361S/P/T/A, T362R, S364A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/T/W/Y/V, S365A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/T/W/Y/V, S366T, S368P/T/A, T369A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/T/W/Y/V, S371Y/H/N/D, S372F/Y/C/L/P/H/R/I/T/N/S/V/A/D/G, T390R, A393R, S394R/P, M398L, S399C/Q/T, Y402F, D403S, S405T, D406N, E408C/R, L410I/R, S411V, A412C, D414A, G447S, S465P.

3. (Unchanged.) A variant of a parent glucoamylase with improved thermostability comprising one or more mutation(s) in the following position(s) or region(s) in the amino acid sequence shown in NO: 2:

Region: 1-18,
Region: 19-35,
Region: 73-80,
Region: 93-127,
Region: 170-184,
Region: 200-212,
Region: 234-246,
Region: 287-319
Region: 334-341,
Region: 353-374,
Region: 388-414.
Region: 445-470,

and/or in a corresponding position or region in a homologous glucoamylase which displays at least 60% homology with the amino acid sequences shown in SEQ ID NO: 2, with the exception of the following substitutions: N20C, A27C, S30P, A246C.

4. (Unchanged.) A variant of a parent glucoamylase with increased specific activity comprising one or more mutation(s) in the following position(s) or region(s) in the amino acid sequence shown in NO: 2:
Region: 1-18,
Region: 40-62,
Region: 93-127,
Region: 170-184,
Region: 200-212,
Region: 234-246,
Region: 287-319,
Region: 388-414,
and/or in a corresponding position or region in a homologous glucoamylase which displays at least 60% homology with the amino acid sequences shown in SEQ ID NO: 2, with the exception of the following substitutions: S411G.

5. (Unchanged.) The variant according to claim 4, having one or more mutation(s) in the following region(s) in the amino acid sequence shown in NO: 2:
Region: 287-300,
Region: 305-319,
and/or in a corresponding position or regions in a homologous glucoamylase which displays at least 60% homology with the amino acid sequences shown in SEQ ID NO: 2.

6. (Amended.) The variant according to [any of claims 1-5] claim 1, wherein the parent homologous glucoamylase is the *Aspergillus niger* G1 glucoamylase.

7. (Amended.) The variant according to [any of claims 1-6] claim 1, wherein the glucoamylase is a truncated glucoamylase, in particular in the C- terminal.

8. (Amended.) A DNA construct comprising a DNA sequence encoding a glucoamylase variant according to [any one of claims 1-7] claim 1.

9. (Unchanged.) A recombinant expression vector which carries a DNA construct according to claim 8.

10. (Amended.) A cell which is transformed with a DNA construct according to claim 8 [or a vector according to claim 9].

11. (Unchanged.) A cell according to claim 10, which is a microorganism, such as a bacterium or a fungus.

12. (Unchanged.) The cell according to claim 11, which is a protease deficient *Aspergillus oryzae* or *Aspergillus niger*.

13. (Amended.) A process for converting starch or partially hydrolyzed starch into a syrup containing dextrose, said process including the step saccharifying starch hydrolyzate in the presence of a glucoamylase variant according to [any of claims 1-7] claim 1.

14. (Unchanged.) The process of claim 14, wherein the dosage of glucoamylase is present in the range from 0.05 to 0.5 AGU per gram of dry solids.

15. (Amended.) The process of [any claims 13 or 14] claim 13, comprising saccharification of a starch hydrolyzate of at least 30 percent by weight of dry solids.

16. (Amended.) The process of [any of the preceding claims] claim 13, wherein the saccharification is conducted in the presence of a debranching enzyme selected from the group of pullulanase and isoamylase, preferably a pullulanase derived from *Bacillus acidopullulyticus* or *Bacillus deramificans* or an isoamylase derived from *Pseudomonas amyloderamosa*.

17. (Amended.) The process of [any of the preceding claims] claim 13, wherein the saccharification is conducted at a pH of 3 to 5.5 and at a temperature of 60-80°C, preferably 63-75°C, for 24 to 72 hours, preferably for 36-48 hours at a pH from 4 to 4.5.

18. (Amended.) A method of saccharifying a liquefied starch solution, which method comprises

(iii) a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent step of

(iv) one or more high temperature membrane separation steps

wherein the enzymatic saccharification is carried out using a glucoamylase variant according to [any of claim 1 to 7] claim 1.